Metagenomic Approaches to Determine Soil Microbial Communities Associated with Armillaria Root Disease

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Introduction

Data was collected at the Priest River Experimental Forest in northern Idaho within a western white pine (*Pinus monticola*) seed provenance study. Six-hundred trees remain from the original 2,400 planted in 1971 (Fig. 1). The research objective is to provide a baseline for soil fungal and bacterial communities, which are associated with two types of *Armillaria* species, *A. solidipes* (high virulence) and *A. altimontana* (low virulence). Determinations of differences in microbial communities can be applied to develop novel management techniques to reduce damage by virulent *Armillaria* species.

Figure 1. A) map of western white pine planting and associated *Armillaria* spp. B) Plot location in northern Idaho.

Materials & Methods

Sampling was completed during late June. From the remaining ca. 600 trees, 63 trees were were selected, based by health status and previous *Armillaria* association. Rhizomorphs, bulk density soil core, DBH, and tree health status were collected from each sampled tree. Soil RNA and DNA were extracted, and tag-amplicon sequencing of the rDNA ITS2 (fungal) and 16S (bacterial) was completed. Rhizomorph-derived cultures were established. DNA was extracted and the translation elongation factor-1α (*tef1*) was amplified and sequenced for species identification. Illumina fastq files were cleaned using Trimmomatic and aligned to Silva and UNITE reference databases for identification. OTU tables were referenced to microbial communities using R. Richness and diversity samples were analyzed.

References

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Results

Species identification found that 56 trees were associated with *A. altimontana*; whereas, only three trees were associated with *A. solidipes*. *A. altimontana* and healthy trees were associated with more diverse bacterial communities, both in richness and Shannon's diversity, compared with *A. solidipes*, less healthy trees, and dead trees; however, this difference was only significant for tree health (Fig. 2 A,C). Interestingly, *A. solidipes* and dead trees were associated with more diverse fungal communities compared to *A. altimontana* and less healthy or healthy trees, although this also was only significant for tree health (Fig. 2 B,D).

Based on the 712 unique bacterial OTUs identified, more Pseudomonadaceae and Spartobacteria were associated with healthy trees, and more Acidobacteria were associated with dead trees (Fig. 3). In respect to *Armillaria* species, more Pseudomonadaceae and Rhizobiales were associated with *A. altimontana*; whereas, more Acidobacteria and Enterobacteriaceae were associated with *A. solidipes* (Fig. 3). Based on the 3,383 unique fungal OTUs identified, more Cortinariceae and Hypocreaceae (*Trichoderma*) associated with healthy trees, but more Inocybaceae were associated with dead trees (Fig. 3). More Trichocomaceae, Cortinariaceae, and Rhizopogonaceae were found in association with *A. altimontana*, and more Mortierellaceae were found in association with *A. solidipes* (Fig. 3).

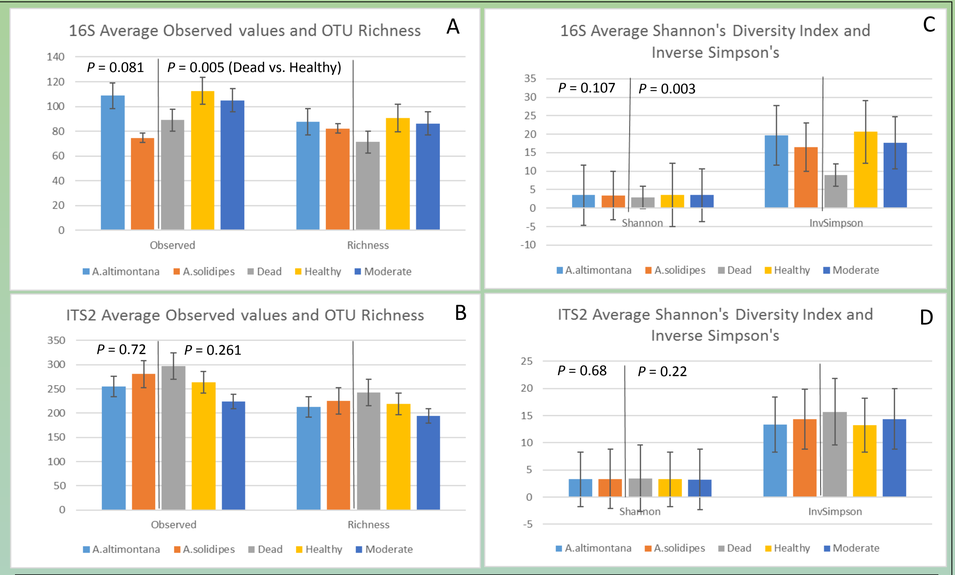

Figure 2. Average observed values and Operational Taxonomic Unit (OTU) richness for bacterial communities (A) and fungal communities (B) Average Shannon's diversity and inverse Simpson's values for bacterial (C) and fungal communities (D).

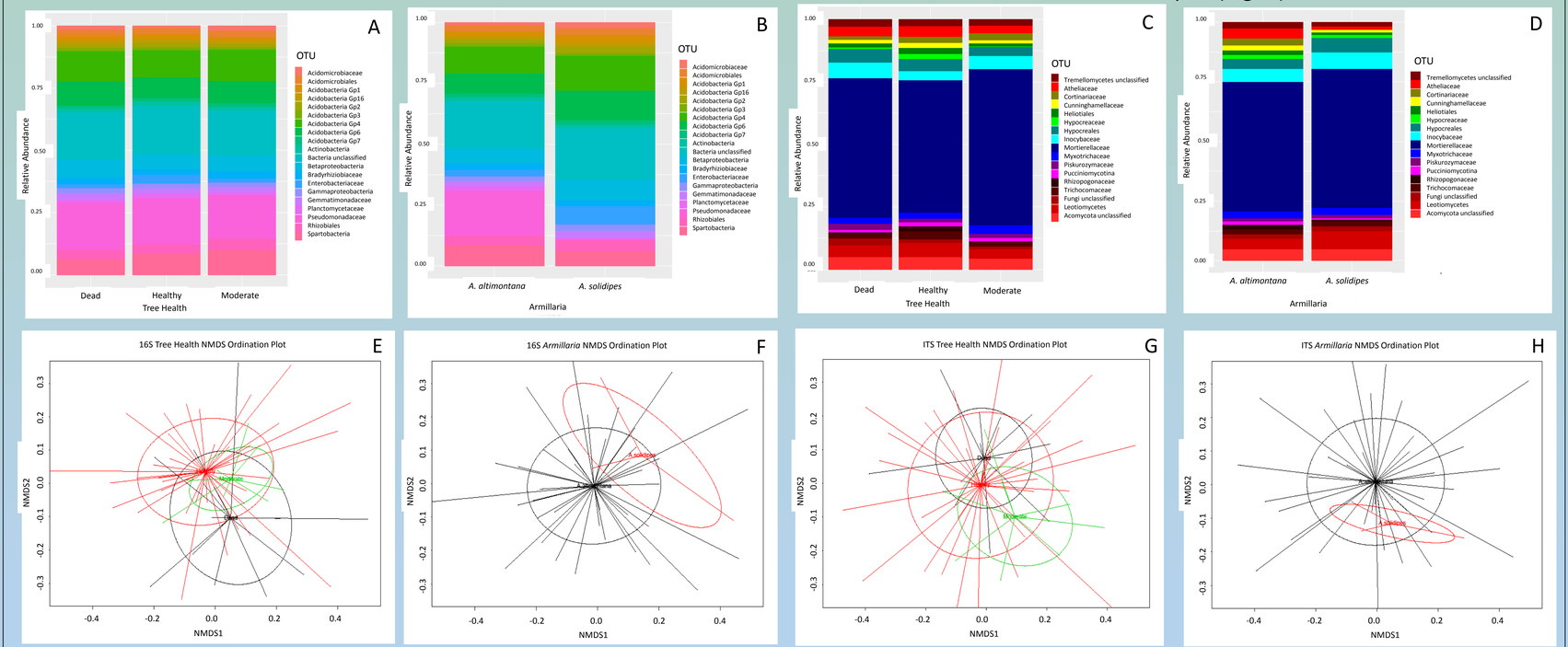

Figure 3. A & B) Stacked bar graph identifying most prevalent bacterial communities; Tree Health (A), *Armillaria* species (B). C & D) Bar graph identifying most prevalent fungal communities; Tree health (C), *Armillaria* species (D). E & F) Ordination plot for bacterial communities ; Tree health (E), *Armillaria* species (F). G & H) Ordination plot for fungal communities; Tree Health (G), *Armillaria* species (H).

Discussion

Potentially higher bacterial diversity is associated with healthy trees and *A. altimontana*; whereas, higher fungal diversity may be associated with dead trees and *A. solidipes.* When examining OTUs within communities, we found higher levels of Pseudomonadaceae and *Trichoderma* species associated with healthy trees and *A. altimontana.* These organisms are known to be important in biocontrol against pathogens in disease-suppressive soils. Preliminary results suggest novel approaches could be developed for managing Armillaria root disease by fostering soil conditions to favor microbial communities that suppress Armillaria root disease. Results will be correlated to soil physical/chemical properties and efforts are underway to replicate these results using artificial inoculations.